# On the Statistical Analysis of Images in Low-Dose Electron Microscopy

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## PHASE CONTRAST IN BIOLOGICAL SPECIMENS

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In the early 1940's, Zernike invented the phase-contrast light microscope[1] as an instrument for imaging "transparent" objects, i.e., objects with a refractive index differing from that of the surrounding medium. A bacterium swimming in water is a good example of such a transparent--or "phase"--object. His revolutionary finding made it possible to see bacteria in their natural environment for the first time.

Scherzer[2] then realised that the wave-aberrations of the electron microscope objective lens were acting in very much the same way as the phase-plate of the optical phase-contrast microscope. The theory of linear transfer of phase information in the electron microscope is now well understood, and the method of (a posteriori) testing the focus of a micrograph in the optical bench with aid of Thon rings[3] can be found in every handbook on electron microscopy.

It may be difficult to accept the idea that an electron microscopical specimen compares to a "glass plate with local variations." Indeed, phase contrast is just one of various contrast mechanisms in the electron microscope, and many of the properties of the microscope, known to the microscopist from daily experience, cannot be described by the phase contrast transfer theory. Phase contrast is, however, the most important source of contrast in structure analysis of biological macromolecules. Moreover, in the new technique of embedding unstained molecules in a layer of vitreous ice[4] the idea of the glass plate is very realistically approximated.

The success of the theory does not mean that it is always well understood by the user of the microscope. In spite of the linearity of the information transfer--for example, artefacts may arise in the image which can easily be misinterpreted. These concepts as well as many others concerning the image formation in the electron microscope may be demonstrated with the aid of computer-simulated images or with optical analogs in an almost standard slide projector.[5]

1. F. Zernike, "A New Method for the Microscopic Observation of Transparent Objects," Part I and II, Physica IX (1942) 686-93, 974-86.
2. O. Scherzer, J. Appl. Phys. 20 (1949) 20-29.
3. F. Thon, "Zur Defokussierungsabhängigkeit des Phasenkontrastes bei der elektronenmikroskopischen Abbildung," Z. f. Naturf. 21a (1966) 476-78.
4. M. Adrian, J. Dubochet, J. Lepault and A.W. McDowall, "Cryo-electron Microscopy of Viruses," Nature 308 (1984) 32-36.
5. M. van Heel, "The Slide Projector as Electron Microscope," Proc. Ninth International Congress on Electron Microscopy, Toronto, 1978, Vol. 1, p. 670.

## ON THE STATISTICAL ANALYSIS OF IMAGES IN LOW-DOSE ELECTRON MICROSCOPY

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An important field of application of electron microscopy is the study of the structure of biological specimens such as proteins. Two major complications arise with the imaging of the substructure of biological objects at high resolution by means of an electron microscope. The first complication is that the quantity related to the object structure is proportional to the phase of an electron wave function which is not directly observable as only intensities can be recorded, for example, on a photographic plate. This so-called phase problem can be overcome by taking two exposures with different imaging conditions; see refs. 1 and 2 for a review on the phase problem. The second complication is that the electrons which interact with the specimen during the image formation inevitably cause irrecoverable structural changes such as, for example, the breaking of chemical bonds. To keep this radiation damage within a prescribed bound, it is necessary to lower the intensity of the electron beam. The electron dose is a compromise between the requirements of minimal radiation damage and sufficient signal-to-noise ratio for subsequent image interpretation. The images become a realization of a stochastic process due to the low electron dose. In this contribution we discuss some aspects of the evaluation of the structural information contained in the images, which are severely degraded by shot noise.

First the image formation and registration is briefly described in a simple model. In order to avoid a complicated formulation, we treat here one lateral dimension of the imaging system only. The extension to two dimensions is trivial in the case of microscopes with square diaphragms. Unfortunately, these microscopes are rarely encountered (if ever) in practice. Once the stochastic properties of the image wave function have been established we will develop techniques for the extraction of information from images of partly unknown specimen structures, i.e., parameter estimation and hypothesis testing. Applications will be presented.

1. H. A. Ferwerda, in: Inverse Source Problems in Optics, ed. H. P. Baltes (Springer-Verlag, Berlin, 1978) chap. 2.
2. C. H. Slump, H. A. Ferwerda, Statistical Analysis of Low-dose Reconstruction of Weak Phase-amplitude Objects from Two Defocused Images, I and II, Optik 62 (1982) 93-104; 143-68.

## QUANTITATIVE ANALYSIS OF MICROSCOPIC IMAGES

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In the past five years considerable changes have occurred in the fields of image processing and pattern recognition. These changes now make it possible to couple sophisticated hardware and software to image acquisition devices such as the electron microscope and to analyze (as well as process) the resulting images. The driving force behind these developments, as in many other technologically based fields, has been a dramatic lowering of costs and an increase in power of computing systems together with a significant improvement in the algorithms for image processing.

The key steps in the quantitative analysis of microscopic images are the following:

1. Image acquisition
2. Image filtering
3. Image segmentation
4. Intra-object measurements
5. Inter-object measurements
6. Analysis of measurements
7. Presentation of results

Each of these steps must be designed in the context of the specific problem as well as the physical constraints of the test specimen and the image scanning device. The sources of noise, image formation, and image distortion, together with the parameters that we desire to measure, will have a strong influence on our choice of image filters (e.g., linear vs. non-linear, binary vs. grey-level), our choice of sampling density (pixels/micron), and our approach to data coding. In certain cases a 10-fold improvement in measurement accuracy has been demonstrated by proper choice of these parameters.

While there exists a reasonable "catalog" of measurements for individual objects (size, shape, texture, "color" etc.), the problem of the quantitative description of inter-object relationships has not been as completely explored; certainly not to the extent of intra-object measures. In addition, the problem posed by the two-dimensional imaging of what are frequently three-dimensional structures demonstrates the need for careful analysis of image parameters using principles of stereology.

## THE LIMITATIONS OF THE CENTRAL NERVOUS SYSTEM AS AN IMAGE AND TEXTURE ANALYZING MACHINE

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As emphasis in morphometry shifts towards serial sectioning analysis, an increasing need for the objective description and classification of morphology will emerge, very likely along the path of "mathematical morphology" being developed by the Fontainbleau group. With all but the simplest geometrical shapes we become rapidly aware of the limitations of our own visual system as an image and texture analyser. This is not surprising, for it has evolved to perpetuate our species in a living world rather than to analyse the shape genera of complex microstructures.

Those involved in image analysis should rightly be interested in the biology of vision and be aware of its limitations. Sensory systems usually modulate their input, and this can be demonstrated by various optical